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ENZYME INDUCTION AND CORTISONE PROTECTION
IN ENDOTOXIN-POISONED MICE AT 25° C
COMPARED WITH THAT AT 5° C

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ABSTRACT

Mice housed at 25° C are protected by cortisone against endotoxin lethality when the hormone is given at the same time as the poison, but not an hour or two later. This is not true of mice housed at 5° C. Activity of liver tryptophan pyrrolase is lowered by endotoxin and elevated by cortisone only in animals at normal temperatures. When endotoxin and hormone are given concurrently, normal enzyme activity is maintained, but activity decreases when the hormone injection is given an hour or more after endotoxin. Actinomycin D, ethionine, 2-thiouracil, and 8-azaguanine (inhibitors of protein synthesis) when given in sublethal amount: potentiate endotoxin, prevent cortisone protection against endotoxin, and block the hormonal induction of tryptophan pyrrolase. Chloramphenicol has none of these effects. Mice infected with Salmonella typhimurium have lower than normal tryptophan pyrrolase activity and a smaller induction of enzyme by cortisone 18 hours postinfection than do normal mice or mice 42 hours postinfection. This occurs only at 25° C.

PUBLICATION REVIEW



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ENZYME INDUCTION AND CORTISONE PROTECTION IN ENDOTOXIN-POISONED MICE AT 25° C COMPARED WITH THAT AT 5° C

SECTION 1. INTRODUCTION

It was suggested in a recent paper of this series (1) that protection by cortisone against the lethal effect of bacterial endotoxin is related to the ability of this and other glucocorticoids to induce de novo synthesis of certain liver enzymes (2, 3, 4). The injection of selected intermediates along the metabolic pathway initiated by the enzyme tryptophan pyrrolase increased survivorship following endotoxin poisoning, indicating that tryptophan pyrrolase plays a protective role in the response of mice to bacterial lipopolysaccharide. It was recognized that other inducible enzymes also might promote reactions of equal or greater importance to the intoxicated animal. One of the significant findings in support of the concept that enzyme induction is involved in an animal's response to endotoxin was the failure of cortisone to induce tryptophan pyrrolase in mice when it was injected four hours after administration of the LD₅₀ of endotoxin (1), or when it was given concurrently to mice housed at 5° C (14). Under the same conditions, cortisone is unable to increase the number of surviving mice. Apparently, therefore, endotoxin is able to block the synthesis of enzyme (protein) stimulated by cortisone.

In agreement with the ability of endotoxin to prevent enzyme induction are observations soon to be published (5) that two known inhibitors of protein synthesis, actinomycin D and ethionine, significantly potentiate endotoxin toxicity in mice when administered in sublethal amounts. These inhibitors also eliminate the protective effect of cortisone against endotoxin lethality.

The experiments described in this report extend these studies — to include additional inhibitors of protein synthesis and to correlate survival data with inducibility of tryptophan pyrrolase.

* This research was conducted in accordance with the "Principles of Laboratory Animal Care" of the National Society for Medical Research.

SECTION 2. METHODS

Endotoxins. Heat-killed Salmonella typhimurium, strain SR-11, was suspended in nonpyrogenic isotonic sodium chloride solution (Baxter Laboratories, Morton Grove, Ill.) and stored at 5° C in screw-capped test tubes, containing about 10 ml per tube. Each ml of suspension contained 6 mg dry weight of bacterial cells. All injections of this material were given intraperitoneally. With each set of experiments, results obtained with heat-killed cells were confirmed with a more highly purified lipopolysaccharide derived from Serratia marcescens.

Inhibitors. Actinomycin D (kindly supplied by Dr. Vernon Bryson, Institute for Microbiology, Rutgers University) was dissolved in 95% ethanol (1 mg/ml) and then diluted in nonpyrogenic saline, so that the desired dose (usually 10 µg per 22 gm mouse) was contained in 0.5 ml. Chloramphenicol was initially dissolved in alcohol and diluted to the desired concentration in nonpyrogenic saline. The other inhibitors were dissolved in 1.5 N sodium hydroxide, diluted with saline, adjusted to pH 8 with hydrochloric acid (as judged by Hydrion test paper) and finally diluted to have the desired amount in 0.5 ml. Saline at pH 8 injected intraperitoneally had no effect on the LD50 of endotoxin. Doses employed were as follows: DL-ethionine (Nutritional Biochemicals, Cleveland), 16 mg per mouse; 2-thiouracil (Mann Research Laboratories, New York), 8 mg per mouse; 8-azaguanine (Nutritional Biochemicals), 8 mg per mouse; and chloramphenicol ("chloromycetin," Parke, Davis and Co., Detroit), 4 mg per mouse. All injections were administered intraperitoneally, and the mice selected weighed 22 ± 1 gm.

Cortisone. Cortisone acetate in stabilized aqueous suspension (United Research Laboratories, Philadelphia) was injected subcutaneously into the interscapular region, 5 mg per mouse contained in 0.5 ml. This is, obviously, a pharmacological dose for mice.

Tryptophan pyrrolase assays. Liver tryptophan pyrrolase was assayed according to the technique of Knox and Auerbach (2) as adapted to mice by Berry and Smythe (1). In all assays, hematin, the cofactor for tryptophan pyrrolase, was added *in vitro* in order to convert all enzyme into holoenzyme, as shown by Feigelson and Greengard (6). For mouse liver, it was experimentally determined that 20 µg of freshly prepared solution per reaction vessel was sufficient. The reaction vessels were incubated in a New Brunswick water bath shaker (New Brunswick Instrument Co., New Brunswick, N. J.) in an atmosphere of pure oxygen for one hour at 37° C. All mice to be used for the assay were fasted at least 17 hours before sacrifice or prior to injection with cortisone, inhibitor, etc.

Protection experiments. In all experiments where mice were to be protected against or sensitized to the lethal effects of bacterial endotoxin, the two injections were given in rapid sequence. When a timed interval of one to four hours was to elapse between injections, 10 animals in a given cage were handled without attempting to keep them in specific order. Survival for a period of 48 hours was taken as permanent survival.

Infection experiments. Mice were infected intraperitoneally with about 10^5 cells of Salmonella typhimurium, strain SR-11, contained in 0.5 ml of a saline dilution of an 18-hour brain-heart infusion broth culture (Baltimore Biological Laboratories, Baltimore, Md.). Dilution was made on the established number of cells such a culture contains, 10^9 per ml. This infectious dose is known to produce first deaths on the third day and to kill all mice after 7 to 10 days.

Statistics. Significance of difference between groups was calculated by the nonparametric rank order test of White (7), the rank correlation method of Wilcoxon (8), or by the chi-square test with Yates' corrected formula (9).

Mice. Female Swiss-Webster mice, weighing 16-18 gm, were purchased weekly from Dierolf Farms (Boyertown, Pa.). After one to two weeks, when they weighed 22 ± 1 gm, they were used experimentally. They were housed 10 per cage in stainless steel cages (some in plastic cages of similar size), with white pine shavings as bedding. Water and pathogen-free mouse food (Dietrich and Gambrill, Frederick, Md.) were available at all times, unless otherwise specified. The animal room and the experimental laboratory were maintained at $25^\circ \pm 2^\circ$ C.

In some of the experiments, mice were given drinking water containing tetracycline antibiotics (Polyotic, American Cyanamid Co., Princeton, N. J.) for two days, on arrival from the dealer. They were then put on tap water for a minimum of a week before they were used experimentally. This treatment was initiated in an effort to improve the reproducibility of results. The antibiotic-treated animals gained weight more rapidly than controls, and experimental findings seem to have been less variable. To the extent of our ability to judge, no undesirable consequences from this procedure were detected.

SECTION 3. RESULTS

Influence of delay on the protective effect of cortisone against endotoxin. Table I summarizes the effect of cortisone on endotoxin lethality when it was given at the same time and one, two and four hours after either the LD₅₀ or twice the LD₅₀ of endotoxin. The well-known protective action of cortisone

TABLE I
EFFECT OF CONCURRENT AND DELAYED INJECTION
OF CORTISONE ON ENDOTOXIN LETHALITY IN MICE

Experimental Treatment	LD ₅₀ Endotoxin		2 X LD ₅₀ Endotoxin	
	Alive Total	P vs. Controls	Alive Total	P vs. Controls
1. Control Mice	$\frac{9}{20}$		$\frac{2}{20}$	
2. 5 mg Cortisone at Time of Endotoxin Injection	$\frac{20}{20}$	<.008	$\frac{15}{20}$	<.008
3. 5 mg Cortisone 1 Hr after Endotoxin Injection	$\frac{16}{20}$.057	$\frac{8}{20}$	N. S. *
4. 5 mg Cortisone 2 Hrs after Endotoxin Injection	$\frac{12}{20}$	N. S.	$\frac{6}{20}$	N. S.
5. 5 mg Cortisone 4 Hrs after Endotoxin Injection	$\frac{11}{20}$	N. S.	$\frac{4}{20}$	N. S.

All injections were given intraperitoneally. Observations were terminated at 48 hours. P-values were calculated by the rank correlation method (8).

* N. S. = Not Significant

is clearly evident against each of the two doses of endotoxin when it was given at the same time as the heat-killed cells (line 2, Table I). Statistically, the degree of protection is approximately the same with each dose of endotoxin. When the hormone injection was given one hour after the endotoxin, statistically borderline protection was afforded mice given the smaller dose but not the larger dose of toxin (line 3, Table I). A delay of either two or four hours between administration of endotoxin and of cortisone eliminated the ability of the hormone to protect the animals against endotoxin, under the conditions of these experiments. It seems clear, therefore, that within an hour or two the mouse was changed by endotoxin in such a way that the metabolic events responsible for survival following an injection of cortisone no longer occurred. The possibility that these metabolic events are either dependent upon or related to the hormone's loss of ability to induce liver enzymes was examined by evaluating the level of tryptophan pyrrolase under conditions comparable to those just described for survival.

Cortisone induction of liver tryptophan pyrrolase after endotoxin. Mice (1) and rats (10) injected with cortisone show a rise in liver tryptophan pyrrolase after a delay of four to six hours. Values for the enzyme derived from control mice and from animals four hours after administration of cortisone are presented in the first two lines of Table II. Under these conditions, the enzyme was about two and one-half times as active in hormone-injected mice as in normal animals. However, if endotoxin was given at the same time as cortisone, there was no significant rise in enzyme activity in the hormone-treated animals (19.4 vs 18.1, lines 3 and 1, Table II). A delay of one or two hours between the injection of cortisone and that of endotoxin permitted the enzyme activity to drop significantly below that seen in normal mice. The data are presented in lines 4 and 5, Table II.

TABLE II

EFFECT OF CONCURRENT AND DELAYED INJECTION OF CORTISONE
ON TRYPTOPHAN PYRROLASE INDUCTION
IN ENDOTOXIN-POISONED MICE

Experimental Treatment	Tryptophan Pyrrolase Activity (μ M Kynurenine/gm Liver/Hr)	
1. Control	18.1 \pm 4.8	(12)
2. 4 Hrs after 5 mg Cortisone	45.6 \pm 6.7	(9)
3. 4 Hrs after 5 mg Cortisone and LD ₅₀ of Endotoxin	19.4 \pm 5.5	(6)
4. 4 Hrs after 5 mg Cortisone and 5 Hrs after LD ₅₀ of Endotoxin	11.5 \pm 4.7	(9)
5. 4 Hrs after 5 mg Cortisone and 6 Hrs after LD ₅₀ of Endotoxin	8.8 \pm 3.2	(9)

Assays were made four hours after cortisone. Animals had been fasted 17-21 hours at time of sacrifice. Each value is the mean \pm standard deviation for the number of determinations shown in parentheses.

On the basis of these observations, it seems clear that cortisone loses its ability to induce tryptophan pyrrolase in the presence of endotoxin, and

indeed, it fails to maintain the normal enzyme level when administered one or two hours after the poison. Thus, under conditions where no enzyme induction occurs in response to cortisone, there is also no increase in survivorship, as the data of Table I established.

Of even greater possible significance to survival is the level of liver tryptophan pyrrolase activity 17 hours after endotoxin. This is the time when mice began to die from the LD₅₀, and it is also the time when the enzyme level was less than one-half normal (line 7 vs. line 1, Table III). Seventeen hours after an injection of cortisone alone, the enzyme activity was nearly triple that for control mice (lines 1 and 2, Table III), while concurrent injection of hormone and endotoxin resulted in a normal level of enzyme activity (line 3). A delay of one, two or four hours in administration of hormone after the injection of endotoxin resulted in a level of tryptophan pyrrolase activity significantly below that for control animals (lines 4, 5 and 6) and an activity only slightly greater than that observed when endotoxin alone was given (line 7). Endotoxin, therefore, under the conditions just described inhibits enzyme induction by cortisone, and the inhibition is evident at both 4 hours (Table II) and 17 hours (Table III) postinjection. At these times after injection of hormone alone, tryptophan pyrrolase was elevated two- to three-fold.

Effect of inhibitors of protein synthesis on lethality of endotoxin. Five different inhibitors of protein synthesis were administered in sublethal amounts to mice, at the same time they received an LD₃₃ of endotoxin. Actinomycin D, ethionine, 2-thiouracil and 8-azaguanine each potentiated the lethal action of endotoxin, with a statistically significant difference from the control value in each case of less than 0.01 as calculated by the chi-square test. Chloramphenicol, on the other hand, failed to alter significantly the number of surviving animals. The last observation is compatible with the finding that chloramphenicol exerts only limited inhibitory action in mammalian systems (11), in contrast to its effect in microbial organisms (12). The other inhibitors, however, are known to act in mammals as well as in bacteria.

Effect of inhibitors of protein synthesis on the protective action of cortisone against endotoxin. If it is assumed that cortisone protects mice against endotoxin lethality because of its ability to induce or maintain synthesis of certain enzymes, then inhibitors of protein synthesis given concurrently should block the protective effect of cortisone. This prediction is confirmed by the data presented in Table V for the same four inhibitors that potentiated the lethality of endotoxin (see Table IV). Chloramphenicol, however, again proved to be the exception, probably because in mice it is not an inhibitor (see Tables VI and VII below).

Effect of inhibitors of protein synthesis on induction of tryptophan pyrrolase by cortisone. If the inhibitors employed in the experiments summarized by the data of Tables IV and V prevent the induction of enzymes

TABLE III

EFFECT OF CONCURRENT AND DELAYED INJECTION OF CORTISONE
ON TRYPTOPHAN PYRROLASE INDUCTION
IN ENDOTOXIN-POISONED MICE

Experimental Treatment	Tryptophan Pyrrolase Activity (μ M Kynurenine/gm Liver/Hr)
1. Control	21.2 \pm 6.0 (7)
2. 17 Hrs after 5 mg Cortisone	54.4 \pm 12.5 (11)
3. 17 Hrs after LD ₅₀ of Endotoxin and 5 mg Cortisone	20.1 \pm 6.2 (20)
4. 17 Hrs after LD ₅₀ of Endotoxin and 16 Hrs after 5 mg Cortisone	10.6 \pm 2.7 (8)
5. 17 Hrs after LD ₅₀ of Endotoxin and 15 Hrs after 5 mg Cortisone	11.0 \pm 6.9 (9)
6. 17 Hrs after LD ₅₀ of Endotoxin and 13 Hrs after 5 mg Cortisone	9.9 \pm 1.5 (8)
7. 17 Hrs after LD ₅₀ of Endotoxin	6.2 \pm 1.5 (8)

Assays were made 17 hours after the endotoxin injection or after cortisone alone. Animals were fasted 17 hours at time of sacrifice. Each value is the mean \pm standard deviation of the number of determinations shown in parentheses.

TABLE IV
**EFFECT OF SUBLETHAL AMOUNTS OF INHIBITORS OF PROTEIN
SYNTHESIS ON LETHALITY OF ENDOTOXIN**

Experimental Treatment	<u>Survivors</u> Total	P vs. Controls
1. 0.75 mg Endotoxin	$\frac{20}{30}$	
2. 0.75 mg Endotoxin + 10 μ g Actinomycin D	$\frac{2}{30}$	< .01
3. 0.75 mg Endotoxin + 16 mg Ethionine	$\frac{6}{30}$	< .01
4. 0.75 mg Endotoxin + 8 mg 2-Thiouracil	$\frac{1}{30}$	< .01
5. 0.75 mg Endotoxin + 8 mg 8-Azaguanine	$\frac{6}{30}$	< .01
6. 0.75 mg Endotoxin + 4 mg Chloramphenicol	$\frac{25}{30}$	N. S. *

All injections were given intraperitoneally at the same time. Observations were terminated at 48 hours, P-values were calculated by the chi-square test (9).

* N. S. = Not Significant

TABLE V

EFFECT OF SUBLETHAL AMOUNTS OF INHIBITORS OF PROTEIN
SYNTHESIS ON THE ABILITY OF CORTISONE TO PROTECT
AGAINST LETHALITY OF ENDOTOXIN

Experimental Treatment	<u>Survivors</u> Total	P vs LPS + Cortisone
1. 3 mg Endotoxin (3 X LD ₅₀)	$\frac{0}{20}$	< .008
2. 3 mg Endotoxin + 5 mg Cortisone	$\frac{12}{20}$	
3. 3 mg Endotoxin + 5 mg Cortisone + 10 µg Actinomycin D	$\frac{0}{20}$	< .008
4. 3 mg Endotoxin + 5 mg Cortisone + 16 mg Ethionine	$\frac{3}{20}$.014
5. 3 mg Endotoxin + 5 mg Cortisone + 8 mg 2-Thiouracil	$\frac{1}{20}$.008
6. 3 mg Endotoxin + 5 mg Cortisone + 8 mg 8-Azaguanine	$\frac{3}{20}$.014
7. 3 mg Endotoxin + 5 mg Cortisone + 4 mg Chloramphenicol	$\frac{14}{20}$	N. S. *

All injections were given intraperitoneally at the same time. Observations were terminated at 48 hours. P-values were calculated by the rank correlation method (8).

* N. S. = Not Significant

by cortisone, then assays for tryptophan pyrrolase should make this apparent. The results presented in Table VI establish such a relationship. Four hours after the injection of cortisone alone, the enzyme level nearly tripled, but when a concurrent injection of actinomycin D, ethionine, 2-thiouracil, or 8-azaguanine was given, liver tryptophan pyrrolase activity was significantly below the control level in each instance. While chloramphenicol significantly lowered the activity of the enzyme below that measured in mice given cortisone alone, it was not lowered to the same level as that observed with the other inhibitors. Of some potential relevance is the fact that all but chloramphenicol potentiated endotoxin and prevented the protective action of cortisone (see Tables IV and V).

Effect of inhibitors of protein synthesis on the ability of cortisone to maintain tryptophan pyrrolase activity in endotoxin-poisoned mice. As stated in an earlier section of this paper, the level of tryptophan pyrrolase in the liver of mice 17 hours after an injection of endotoxin is believed to be a more reliable indication of the severity of the poisoning than values obtained after 4 hours. Accordingly, the experiments summarized in Table VII were undertaken. Examination of the results makes it clear that endotoxin alone lowered tryptophan pyrrolase to about one-third the control level (lines 1 and 2). Cortisone alone raised the activity two- to three-fold (line 3). When the two were combined, the enzyme remained at the control level (line 4), while the added injection of actinomycin D, ethionine, 2-thiouracil or 8-azaguanine resulted in the same suppression of tryptophan pyrrolase as that observed with endotoxin alone (lines 5-8). Chloramphenicol, however, produced no change in enzyme activity when it was injected concurrently with both endotoxin and cortisone, since the level of enzyme was almost identical to that found when the antibiotic was not given (lines 9 and 4). Therefore, on the basis of the data of Table VII, it seems permissible to conclude that the inhibitory effect of chloramphenicol is less intense and prolonged than that of the other inhibitors, and its inability to alter the lethality of endotoxin in either the presence or the absence of cortisone, may be tentatively explained in this way.

Induction of tryptophan pyrrolase by cortisone in mice infected with *Salmonella typhimurium*. If it is assumed that mice experimentally infected with a Gram negative pathogen, such as *Salmonella typhimurium*, strain SR-11, undergo metabolic alterations in part attributable to release of endotoxin, then one might predict for such animals an impairment in cortisone induction of liver tryptophan pyrrolase. Partial confirmation of this prediction is given in the results presented in Table VIII. The level of enzyme was significantly lower 18 hours postinfection than in uninfected control animals (lines 1 and 2). At the same time postinfection, cortisone caused an increase in the level of enzyme activity, but the absolute augmentation was not as high as that for control mice (line 1) or for mice 42 hours postinfection (line 3). Thus both the lower control value for tryptophan pyrrolase and the smaller induction with cortisone in mice 18 hours postinfection are consistent with

TABLE VI

EFFECT OF INHIBITORS OF PROTEIN SYNTHESIS ON CORTISONE
INDUCTION OF LIVER TRYPTOPHAN PYRROLASE IN MICE

Experimental Treatment	Tryptophan Pyrrolase Activity (μ M Kynurenine/gm Liver/Hr)	
1. Control Mice	18.1 \pm 4.8	(12)
2. 4 Hrs after 5 mg Cortisone	45.6 \pm 6.7	(9)
3. 4 Hrs after 5 mg Cortisone + 10 μ g Actinomycin D	27.4 \pm 5.0	(12)
4. 4 Hrs after 5 mg Cortisone + 16 mg Ethionine	9.6 \pm 3.7	(8)
5. 4 Hrs after 5 mg Cortisone + 8 mg 2-Thiouracil	17.8 \pm 4.5	(7)
6. 4 Hrs after 5 mg Cortisone + 8 mg 8-Azaguanine	27.9 \pm 5.9	(7)
7. 4 Hrs after 5 mg Cortisone + 4 mg Chloramphenicol	35.2 \pm 6.0	(8)

Assays were made four hours after cortisone. Animals had been fasted 17-21 hours at time of sacrifice. Each value is the mean \pm standard deviation for the number of determinations shown in parentheses.

TABLE VII
EFFECT OF ENDOTOXIN AND INHIBITORS OF PROTEIN SYNTHESIS
ON CORTISONE INDUCTION OF LIVER TRYPTOPHAN
PYRROLASE IN MICE

Experimental Treatment	Tryptophan Pyrrolase Activity (μ M Kynurenine/gm Liver/Hr)
1. Control Mice	21.2 \pm 6.0 (7)
2. Endotoxin (LD ₅₀)	6.2 \pm 1.5 (8)
3. Cortisone (5 mg)	54.4 \pm 12.5 (11)
4. Endotoxin + Cortisone	20.1 \pm 6.2 (16)
5. Endotoxin + Cortisone + Actinomycin D (10 μ g)	5.6 \pm 1.2 (7)
6. Endotoxin + Cortisone + Ethionine (16 mg)	5.5 \pm 2.6 (7)
7. Endotoxin + Cortisone + 2-Thiouracil (8 mg)	5.6 \pm 2.1 (6)
8. Endotoxin + Cortisone + 8-Azaguanine (8 mg)	7.5 \pm 3.0 (7)
9. Endotoxin + Cortisone + Chloramphenicol (4 mg)	20.0 \pm 9.2 (6)

Assays were made 17 hours after the injections, all given at the same time. Animals had been fasted 17 hours at time of sacrifice. Each value is the mean \pm standard deviation for the number of determinations shown in parentheses.

TABLE VIII

EFFECT OF INFECTION WITH S. TYPHIMURIUM, SR-11,
ON CORTISONE INDUCTION OF LIVER TRYPTOPHAN PYRROLASE

Experimental Treatment	Tryptophan Pyrrolase Activity (μ M Kynurenine/gm Liver/Hr)			
	4 Hrs after Injection of Saline		4 Hrs after Injection of 5 mg Cortisone	
Controls (No Infection)	18.1 \pm 4.8	(12)	45.6 \pm 6.7	(9)
18 Hrs Postinfection	13.8 \pm 4.3	(20)	29.1 \pm 8.0	(10)
42 Hrs Postinfection	25.2 \pm 8.1	(6)	55.8 \pm 3.3	(6)

Assays were made four hours after cortisone at indicated times postinfection. Each value is the mean \pm standard deviation for the number of determinations shown in parentheses.

the presence of endotoxin. Earlier results from this laboratory (13) also showed that mice with experimental salmonellosis behave as if endotoxin is present during the first day postinfection but not during the second day, under conditions comparable to those used in the present study. Quite different assays for endotoxin were employed in the earlier work. Until more is known about the requirements for enzyme induction in mammalian species, a conservative interpretation of these findings with infected animals is imperative.

SECTION 4. DISCUSSION

The results described in this report, when combined with work already published (1) or in press (5, 14), make it seem undeniably clear that: endotoxin lowers the activity of liver tryptophan pyrrolase; cortisone raises it; and when the two are administered at the same time, a normal level of activity is maintained. There are several conditions under which cortisone fails to increase the survival of endotoxin-poisoned mice. The best-known situation is when the hormone injection is delayed for a few hours after the administration of endotoxin, but to be added to this is the inclusion of an inhibitor of protein synthesis and exposure to a stressful temperature of 5° C (14). Under all of these conditions, the common denominator seems to be failure to induce or maintain activity of tryptophan pyrrolase. While this is, at present, the only enzyme studied in this connection, we do not intend to suggest that it is the only one of importance. Other inducible enzymes, including those important in gluconeogenesis (15, 16, 17), may also be involved in some of the metabolic alterations previously described and now associated with endointoxication (18, 19). This is especially true of the depletion in glycogen reserves in muscle and liver and the hypoglycemia that characteristically accompanies injection of toxic doses of endotoxin.

The promptness with which inducible liver enzymes under-increase or decrease following injection, respectively, of cortisone or inhibitor of protein synthesis (such as ethionine) is probably a reflection of their half-lives; at least, this is the interpretation advanced by Goldstein et al (20) for liver tryptophan pyrrolase in rats. From this point of view, tryptophan pyrrolase has a shorter half-life than the enzymes responsible for gluconeogenesis, the latter as judged by the data of Greengard et al (16) and of Weber et al (17) in rats. That the sequence of metabolic changes following an injection of endotoxin may be dependent partly on relationships of this type is a concept that lends itself to direct experimental study.

One of the major questions remaining to be resolved is the primary effect and/or site of action of endotoxin. From the results presented above, it takes an hour or more for an LD₅₀ to produce demonstrable inhibition of

inducible enzymes. When this is balanced against the survival time of 18-36 hours for mice injected with this amount of endotoxin, it is possible to detect events that occur in 5% or less of the time required for the total sequence of changes leading to death. However, this is longer in time than the reported release of lysosomal enzymes in livers of rats given a dose of endotoxin of comparable magnitude. The group at New York University (21) detect significant changes within as little as five minutes of an injection. This is dramatically rapid, and one must consider that the cytological alterations accompanying intracellular exposure to the various hydrolases from lysosomes could soon produce, certainly within an hour, a block in inducibility of hepatic enzymes. Unfortunately, it is impossible at the present time to test unambiguously such an interpretation of sequential changes that follow endotoxin injection. The need for future investigations along these lines hardly requires comment.

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